Cause-specific mortality data on Danish monozygotic and dizygotic twins are used to analyze heritability estimates of susceptibility to coronary heart disease (CHD) after controlling for smoking and Body Mass Index (BMI). The sample includes 1209 like-sexed twin pairs born between 1890 and 1920, where both individuals were still alive in 1966. The participants completed a questionnaire in 1966 which included questions on smoking, height and weight. The analysis was conducted with both sexes pooled due to the relatively small number of twin pairs. Follow-up was conducted from January 1, 1966 to December 31, 1993. The correlated gamma-frailty model with observed covariates was used for the genetic analysis of frailty to account for censoring and truncation in the lifetime data. During the follow-up, 1437 deaths occurred, including 435 deaths due to CHD. Proportions of variance of frailty attributable to genetic and environmental factors were analyzed using the structural equation model approach. Different standard biometric models are fitted to the data to evaluate the magnitude and nature of genetic and environmental factors on mortality. Using the best-fitting model without covariates, heritability of frailty to CHD was found to be 0.45 (0.11). This result changes only slightly to 0.55 (0.13) in a DE model after controlling for smoking and BMI. This analysis underlines the existence of a substantial genetic influence on individual frailty associated with mortality caused by CHD.

Twin studies are one of the most widely used methods for quantifying the influence of genetic and environmental factors on specific diseases. In the case of binary traits (where disease is either present or not), concordance analysis provides a powerful and widely accepted method in genetic epidemiology. Concordance rates are easy to calculate and allow a clear interpretation (Gatz et al., 2000; McGue, 1992). In practical applications, time-to-event data (time of onset of disease, age at death) is often available, but usually in a truncated and/or censored form. Censoring of bivariate observations can be a complex problem as either or both individuals of a pair may be subject to censoring, and the censoring times need not be the same for both individuals. Additional problems arise through bivariate truncation, which implies a nonrandom selection of the study population from the total twin population. Furthermore, in many cases covariates are available. Unfortunately, it is difficult to manage truncated and censored time-to-event data and covariates within the context of concordance analysis. A large part of the motivation for the methodology of this article is exploring the potential for censored and truncated data and the inclusion of measured covariates. One key question here is whether the inclusion of covariates changes the heritability estimates of susceptibility to the disease under study.

We aim to study bivariate survival times $T_1$, $T_2$ which depend, via proportional hazards models, on unobserved variables $Z_1$, $Z_2$ (called frailties). That is, we seek to explain an association between two (non-negative) survival times. They have a continuous joint distribution through their common dependence upon unobserved random variables. Such models are particularly convenient in the context of survival data and they stem from the (univariate) concept of frailty introduced by Vaupel and colleagues (Vaupel et al., 1979). The univariate frailty model extends immediately to frailty models with a bivariate survival function $S(t_1, t_2) = P(T_1 > t_1, T_2 > t_2)$. The bivariate frailty model asserts that $T_1$ and $T_2$ are conditionally independent, given $Z_1$, $Z_2$, and that

$$S(t_1, t_2) = E(e^{-Z_1 \Phi(t_1) - Z_2 \Phi(t_2)})$$
for some cumulative baseline hazard functions $H_{01}$ and $H_{02}$. We will give more detailed information about this model presently.

We want to apply the bivariate frailty model to twin data on mortality due to coronary heart disease (CHD). The role of family aggregation of CHD is well established. Questions about the nature of the genetic effects (additive vs. nonadditive) are addressable. Former studies from the Danish (Harvald & Hauge, 1970) and Swedish twin registries (de Faire et al., 1975; Marenberg et al., 1994) found a genetic component in the risk of death from coronary heart disease. Recent studies (Wienke et al., 2001; Zdravkovic et al., 2002) have used approaches from survival analysis to account for truncation and censoring present in the data. Such methods have to be combined with genetic epidemiology methods. The heterogeneity of individuals with susceptibility to CHD as well as important covariates are included in the present model. For such a combined analysis, the correlated gamma-frailty model with observed covariates (Yashin et al., 1996; Yashin & Iachine, 1997) was applied in this paper, which takes into account the dependence of life spans of relatives (twins). This approach enables the combination of age at death data with data on the cause of death, smoking, and Body Mass Index (BMI); and further deals with truncated and censored observations. Individuals who died from causes other than CHD were considered as censored and combined with the censored individuals who did not die during the follow-up period. The advantages of the model were empirically demonstrated in the statistical analysis of lifetime data from Danish twins that were used by Herskind, McGue, Iachine and colleagues (1996), but with special focus on mortality caused by CHD and the application of a frailty model.

Materials and Methods

Twins' mortality data were provided by the Danish Twin Registry, founded in 1954 as the world's first nationwide twin registry. This population-based registry includes twins born in Denmark during the period 1870 to 1910 and all like-sex pairs born between 1911 and 1930. For detailed information about the Danish Twin Registry, see Hauge (1981).

In 1966, a questionnaire including questions about smoking, height and weight was mailed to all twins born between 1890 and 1920 who were alive and traceable on January 1, 1966 — 3709 individuals answered the questionnaire (response rate 65%). Questions about phenotypic similarities included in the questionnaire were used to assess the zygosity. This zygosity classification was compared with laboratory methods (serological markers). The misclassification rate was found to be below 5% (Holm, 1983; Lykken, 1978). Excluded from the study were 813 twins with nonresponding partners, four pairs with unknown zygosity and 212 pairs with incomplete or uncertain information on height and weight. Twenty-three pairs were excluded because of incomplete information about cause of death, resulting in a study population of 1209 twin pairs.

Smoking status was divided into four groups: nonsmokers, ex-smokers, current cigar or pipe smokers and current cigarette smokers. Nonsmokers were defined as individuals who had smoked less than 100 cigarettes, 50 cigars or cigarillos, or 5 packs of tobacco in their lifetime. Persons who gave no information on lifetime smoking status or current smoking status were pooled with the ex-smokers. Current cigarette smokers who also smoked other kinds of tobacco were included in the group of current cigarette smokers. As the association between BMI and CHD mortality is J- or U-shaped rather than linear, BMI was divided into three groups: < 22 kg/m$^2$, 22–28 kg/m$^2$, and > 28 kg/m$^2$.

Individuals were followed from January 1, 1966 to December 31, 1993. Those persons identified as deceased after that date are classified as living for our purposes. At the end of the follow-up, approximately 40% of the twins were still alive, resulting in right censored data. Two hundred and ten male monozygotic (MZ) and 316 dizygotic (DZ) twin pairs, and 273 female MZ and 410 DZ twin pairs participated. In addition to age at death, information on cause of death was also available for all individuals who died during the follow-up. For the study, only the underlying cause of death was considered. Detailed information about death status, gender, zygosity, smoking, and BMI of the study population is given in Table 1 and 2.

| Study Population (Number of Individuals) by Gender, Zygosity and Cause of Death |
|------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Males | Females | Males | Females | Males | Females |
| Deaths | | | | | | |
| CHD | | | | | | |
| Other | | | | | | |
| All causes | | | | | | |
| Alive | | | | | | |
| Total | | | | | | |

Table 1

Table 2

Mortality

After the age of 6 years, death rates for Danish twins born between 1870 and 1900 are almost the same as those for the same cohorts of the Danish population. The distributions of age at death for MZ twins are close to those of DZ twins for both sexes (Christensen et al., 1995; Christensen et al., 2001). Recent papers dealing with twin cohorts born during the period 1870 to 1930 found similar mortality patterns for Danish twins and the general Danish population with respect to CHD (Christensen et al., 2001; Wienke et al., 2001).
the frailties, and \(X_j\) of dying as a function of age and BMI. Additionally, a decomposition of frailties \(Z_1 = \text{twin partners be conditionally independent given their environmental factors acting on susceptibility (frailty) to mortality due to CHD are analyzed when smoking and BMI are controlled for. The correlated gamma-frailty model with observable covariates can be used to fit bivariate lifetime data and to provide a specific parameter for correlation of frailty to death. It is needed that allows indirect calculation of the parameters. The bivariate gamma distributed frailty with the above-mentioned properties was constructed in Yashin et al. (1995). The unconditional bivariate survival function is given by:

\[
S(t_1, t_2 | X_1, X_2) = \frac{S(t_1 | X_1)^\gamma S(t_2 | X_2)^\gamma}{(S(t_1 | X_1)^\gamma + S(t_2 | X_2)^\gamma - 1)^{\gamma/\rho}}
\]

where \(S(t|X)\) denotes the marginal univariate survival function, assumed to be equal for both partners in a twin pair. Using a parametric approach a Gama-Gompertz model was fitted to the data, for example, \(S(t | X) = (1 + [(1 + s^2 \alpha(e^\gamma - 1))e^\beta t]^\gamma^{-1})e^{-\rho t}\)

where \(\alpha, \gamma, s^2, \sigma^2, \rho, \beta\) are parameters to be estimated.

The lifetimes \(T_{i1}, T_{i2}\) are assumed to be independently censored from the right. Starting from this model, the likelihood function of the data is able to be derived:

\[
L(t_1, t_2, \delta_1, \delta_2) = \delta_1 \delta_2 \sum_{i=1}^{n_1} \left[ -\frac{S(t_1 | X_i, X_j) - \delta_i (1-\delta_1) S_{i1}}{(1-\delta_1) S_{i1}(1-\delta_2) S_{i2}} \right]
\]

with \(\delta_i (i = 1, ..., n; j = 1, 2)\) as censoring indicator. Partial derivatives of the marginal survival functions are given by

\[
S_{ii}(t_i, t_j | X_i, X_j) = \frac{\partial^2 S(t_i, t_j | X_i, X_j)}{\partial t_i \partial t_j}
\]

and

\[
S_{ij}(t_i, t_j | X_i, X_j) = \frac{\partial^2 S(t_i, t_j | X_i, X_j)}{\partial t_i \partial t_j}
\]

Because of the independence assumption between lifetimes and censoring times, the distribution of the censoring times does not enter the likelihood function.

As mentioned above, the twin pair data set used is not randomly selected from the total twin population. Since both members of a twin pair had to be still alive.
on January 1, 1966, the survival times in the data set are sampled from specific conditional distributions. If a twin pair was born in year 1880 (or 1881, ..., 1920), the condition of survival of both twins until the year 1966 implies that both twins had to survive until the age of $t^* = 1966 - 1880$ in order to be included in the sample. If the survival times are denoted by $T_1$ and $T_2$ with survival function $S(t, t_1 | X_1, X_2)$, then the conditional survival function for a twin pair is:

$$S(t_1, t_2 | X_1, X_2, T_1 > t^*, T_2 > t^*) = \frac{S(t_1, t_2 | X_1, X_2)}{S(t^*, t^* | X_1, X_2)}$$

Consequently, the likelihood function of independently left truncated and right censored lifetime data is given by:

$$L(t_1, t_2, \delta_1, \delta_2, t^*) = \frac{\delta_2 S(t_1, t_2 | X_1, X_2) - \delta_1 (1 - \delta_1) S(t_1, t_2 | X_1, X_2)}{S(t^*, t^* | X_1, X_2)} - (1 - \delta_1) \delta_2 S_t(t_1, t_2 | X_1, X_2) + (1 - \delta_1) (1 - \delta_2) S(t_1, t_2 | X_1, X_2)$$

For a combined analysis of MZ and DZ twins, two correlation coefficients $\rho_{MZ}$ and $\rho_{DZ}$ were included respectively. The correlations between MZ and DZ twins provide information about genetic and environmental influences on frailty within individuals.

### Quantitative Genetics of Frailty

In studies of MZ and DZ twin pairs, the intrapair-correlations of the trait under study (in this case frailty on mortality due to CHD) play the key role for analysis of genetic and environmental factors. Using these coefficients, five standard genetic models of frailty are fitted to the data that correspond to five different assumptions about its structure. Resemblance in twins (completely for MZ twins and partly for DZ twins) is caused by three factors: additive genetic factors (A), genetic factors due to dominance (D) and shared environmental factors (C). Nonshared environment (completely for MZ twins and partly for DZ twins) is responsible for intrapair differences in twins. From the estimation point of view, three parameters could be included into the model simultaneously as there are data from two different groups of relatives (MZ and DZ twins). Models that are more complex need data from additional groups of relatives. Each additional group allows for an additional parameter in the model. The following biometric models were fitted to the data: ACE, ADE, AE, DE and CE. In these notations, an ACE model refers to the decomposition of frailty $Z = A + C + E$. ADE, AE, DE and CE models are defined similarly. Small letters $a^2$, $c^2$, $e^2$, $d^2$ are used to refer to the respective proportions of variance. For example, the relation $1 = a^2 + c^2 + e^2$ corresponds to the decomposition of variance in the ACE model of frailty. Standard assumptions about of the quantitative genetics yields in the following relations:

$$\rho_{MZ} = a^2 + d^2 + c^2$$
$$\rho_{DZ} = 0.5a^2 + 0.25d^2 + c^2$$

Here $a^2 + d^2$ denotes (broad sense) heritability of the trait under study. For detailed information about these genetic models and deriving the upper equations, see the monograph by Neale and Cardon (1992). Note that the AE model and the CE model are not nested. Consequently, the likelihood ratio test could not be used to define the model with the best fit to the data. The Akaike Information Criterion (AIC; Akaike, 1987) is used to compare nonnested models.

To combine the approach of quantitative genetics with the methods of survival analysis the correlated gamma-frailty model is used with genetic and environmental components of frailty. In this approach, the decompositions in [2] must be substituted into survival model [1]. This model must be used to estimate the parameters $a^2$, $d^2$, $c^2$, $e^2$ by the maximum likelihood method directly. Analysis was made using the standard statistical software package GAUSS.

### Results

As not all models are nested, the likelihood ratio test can not be applied to compare all five biometric models. Applying the correlated gamma-frailty model with and without observed covariates, the Akaike Information Criterion prefers the AE and DE model respectively. There is not much difference in the fit between the AE and DE model. Using these two models, heritability changes from 0.43 (0.11) without covariates to 0.48 (0.12) with covariates (AE model) and from 0.50 (0.13) in the model without covariates to 0.55 (0.13) in the model with covariates (see Table 3). Standard errors for the ACE model are not shown in Table 3 since $c^2 = 0$ is the boundary of the parameter space.

Using the best-fitting DE model, the likelihood ratio test indicates a significant influence of BMI on CHD mortality ($\beta_1 = 0.53 \pm 0.25$ and $\beta_3 = 0.47 \pm 0.24$). Here $\beta_1$ and $\beta_3$ describe individuals with BMI less than 22 kg/m$^2$ and more than 28 kg/m$^2$ respectively. The reference group are individuals with BMI between 22 kg/m$^2$ and 28 kg/m$^2$. Smoking shows a significant influence on CHD mortality ($\beta_6 = 0.57 \pm 0.24$, $\beta_7 = 0.32 \pm 0.23$ and $\beta_8 = 0.48 \pm 0.26$). Here $\beta_6$, $\beta_7$ and $\beta_8$ denote cigarette smokers, pipe/cigar smokers and former smokers respectively. The reference group is the nonsmokers. $\beta_6$ denotes the risk of males compared to females. The results with respect to the (second best) AE model are similar.

### Discussion

The method presented in this article with its suitability for censored and truncated data and the possibility to include observed covariates overcomes the well-known drawbacks of the traditional concordance analysis in twin studies with time-to-event data. An important question arising from the genetic analysis...
of models with observed covariates (in this case smoking and BMI) is whether genes that are responsible for variation in observed covariates also contribute to a variation in susceptibility to CHD. Traditional biometrical methods of regression analysis can lead to spurious effects of covariates. In extreme cases, common genes not covariates may be responsible for variation in life span.

Both smoking and BMI are influenced by genes with heritability estimates 0.35–0.75 (smoking) and 0.5–0.8 (BMI; Bouchard, 1994; Heath & Madden, 1995; Herskind, McGue, Sørensen et al., 1996). Whether common genes influence these phenotypic traits as well as susceptibility to CHD, however, is an open question. In 1958, Fisher suggested that the association between smoking and lung cancer is spurious and reflects only the circumstance that the same genes influence both smoking habits and lung cancer. This was the starting point for a long debate on genetic confounding.

The conclusion of this paper is that the inclusion of smoking and BMI do not cause any substantial changes in the heritability estimates. Hence, no evidence was found for common genetic factors acting on smoking and susceptibility to CHD, or BMI and susceptibility to CHD. This study confirms the earlier finding by Zdravkovic et al. (2004) that the genetic influence on susceptibility to CHD is not mediated through genetic influence on smoking and BMI.

As expected, the inclusion of observable covariates decreases the heterogeneity in the study population that can be seen in the decline of variance in frailty from $\sigma^2 = 7.74$ to $\sigma^2 = 4.54$ (AE model) and from $\sigma^2 = 7.16$ to $\sigma^2 = 4.28$ in the DE model. It is therefore clear that frailty is not a phenotypic trait. Frailty depends on the model; it describes factors not included in the model.

When observed covariates are included in the model, the relative importance of environmental factors (shared environment, C, and nonshared environment, E) is reduced, leading to an increase in the heritability estimates observed in the present analysis. This may appear strange, although use of the heritability coefficient when computed as a proportion of variance may be misleading. The variance in the trait can be decomposed as follows: $\sigma^2 = \sigma^2_{\text{genes}} + \sigma^2_{\text{environment}}$.

One does not know whether the heritability increases due to an increase in the genetic variance or due to a decrease in the environmental variance. In the present case, the (moderate) increase was largely due to the influences of the latter. By including smoking and BMI, the genetic variance was reduced from $\sigma^2_{\text{genes}} = 3.48$ to $\sigma^2_{\text{genes}} = 2.18$ in the AE model and from $\sigma^2_{\text{genes}} = 3.58$ to $\sigma^2_{\text{genes}} = 2.35$ in the DE model respectively. The reduction of the environmental heterogeneity in frailty is more pronounced, for example, from $\sigma^2_{\text{environment}} = 4.26$ to $\sigma^2_{\text{environment}} = 2.36$ in the AE model and from $\sigma^2_{\text{environment}} = 3.58$ to $\sigma^2_{\text{environment}} = 1.93$ in the DE model. Thus, the focus should not be on the increase in heritability but on the decrease in environmental variance.
rather on the decrease in the environmental variance. These results help us understand that the risk factors studied represent primarily environmental sources of variation for CHD-death, despite the role that genetic influences may play in specific risk factors. For a more detailed discussion of variance components, see Hopper (1993).

This point is also important when reviewing why the AE model could be replaced by the DE model when observed covariates are taken into account. For example, this could occur when the additive genetic component of observed covariates contains a substantial portion of the additive genetic component of a trait (as well as a part of independent environmental component). The resulting genetic and environmental variances of the trait may be reduced substantially as in this case. The sizes of these parts will determine the best-fitting model and the new value of heritability estimate. It may well be then that the residual proportion of genetic variation in the trait (frailty) in the presence of observed covariates is mostly due to dominant genetic effects.

The analysis underlines the importance of genetic factors on individual susceptibility to CHD. The heritability estimate in this study (0.45 for both sexes combined in an AE model without covariates) is lower than those found in a previous analysis of an extension of the presented data set (without covariates) with heritability estimates of 0.53 and 0.58 for males and females respectively (Wienke et al., 2001). The lower estimates in this subsample may be a consequence of a decline in the heritability of CHD with increasing age as found in Marenberg et al. (1994) and Zdravkovic et al. (2002). The twin population in this study is much older, the time of truncation was 1966 compared with 1943 in the paper by Wienke et al. (2001). Furthermore, the youngest cohorts (birth years 1921 to 1930) are not included in the present analysis.

The main limitation of the present study is that the risk factor information was obtained from a self-report questionnaire in 1966. It cannot be ruled out that self-reported information, especially regarding weight, is biased downwards. Smoking information appears reliable, as smoking was widely accepted in the 1960s. Alternatively, people reported as smokers in the 1960s may have since stopped smoking, but still be in the smoking category, which may result in a downward risk bias for smokers. Detailed information about tobacco smoked per day over the follow-up period is not available. Consequently, the group of smokers consists of both heavy and light smokers, which may result in an additional downward risk bias for smokers.

The proposed method allows the censored and truncated time-to-event data handling of related individuals in the case of observed covariates. The hypothesis of genetic confounding can be checked and the influence of observable covariates on heritability analyzed.

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References


